

**This Page Is Inserted by IFW Operations
and is not a part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ³ : C07D209/58; A61K 31/40		A1	(11) International Publication Number: WO 84/ 01382 (43) International Publication Date: 12 April 1984 (12.04.84)		
(21) International Application Number: PCT/US83/01379 (22) International Filing Date: 9 September 1983 (09.09.83)		(74) Agents: FETZER, Robert, J. et al.; Baldwin, Egan, Walling & Fetzer, 607 Hanna Building, Cleveland, OH 44115 (US).			
(31) Priority Application Numbers: 424,647 481,345		(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, FR (European patent), GB (European patent), JP, LU (European patent), NL (European patent), NO, SE (European patent).			
(32) Priority Dates: 27 September 1982 (27.09.82) 1 April 1983 (01.04.83)					
(33) Priority Country: US		Published <i>With international search report.</i>			
(71) Applicant: ONCOLOGY RESEARCH & DEVELOPMENT, INC. [US/US]; 3348 Genesee Street, Cheektowaga, NY 14225 (US).					
(72) Inventors: WEISHAUPP, Kenneth, R. ; 94 Rutland Avenue, Sloan, NY 14212 (US). DOUGHERTY, Thomas, J. ; 2306 West Oakfield, Grand Island, NY 14072 (US). POTTER, William, R. ; 1876 East River Road, Grand Island, NY 14072 (US).					
(54) Title: PURIFIED HEMATOPORPHYRIN DERIVATIVE FOR DIAGNOSIS AND TREATMENT OF TUMORS, AND METHOD					
(57) Abstract					
<p>A method of synthesizing and purifying a high molecular weight aggregate, derived from hematoporphyrin, said material being useful in the localization and treatment of tumors. The substance results from the hydrolysis of the reaction mixture of hematoporphyrin and acetic-sulfuric acids. The material is purified by cycling the crude product through a micro-porous membrane system in which exclusion of low molecular weight by-products is effected. The substance is tumor-selective and photosensitizing. It is retained in tumors longer than in normal tissues and fluoresces red to aid in diagnosis. Strong illumination of tumors retaining the substance results in necrosis, while normal tissues which have generally eliminated the drug remain unaffected. The high molecular weight aggregate has an empirical formula of approximately C₆₈H₇₀N₈O₁₁ (Na plus H)₄.</p>					

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	LI	Liechtenstein
AU	Australia	LK	Sri Lanka
BE	Belgium	LU	Luxembourg
BR	Brazil	MC	Monaco
CF	Central African Republic	MG	Madagascar
CG	Congo	MR	Mauritania
CH	Switzerland	MW	Malawi
CM	Cameroon	NL	Netherlands
DE	Germany, Federal Republic of	NO	Norway
DK	Denmark	RO	Romania
FI	Finland	SE	Sweden
FR	France	SN	Senegal
GA	Gabon	SU	Soviet Union
GB	United Kingdom	TD	Chad
HU	Hungary	TG	Togo
JP	Japan	US	United States of America
KP	Democratic People's Republic of Korea		

-2-

26:1-8, 1961, Lipson and co-workers disclosed a crude material, prepared by acetic acid-sulfuric acid treatment of hematoporphyrin, said material having a superior ability to localize in tumors.

- 5 The photosensitive characteristic of tumor-selective porphyrin compounds also make them useful in the treatment of tumors. In "Photodynamic Therapy of Malignant Tumors", Lancet 2:1175-1177, 1973, Diamond and Co-workers achieved tumor necrosis after lesion-
10 bearing rats were injected with hematoporphyrin and exposed to white light. In "Photoradiation Therapy for the Treatment of Malignant Tumors", Cancer Res. 38:2628-2635, 1978 and "Photoradiation in the Treatment of Recurrent Breast Carcinoma", J Natl Cancer Inst. 62:231-237, 1979, Dougherty and co-workers reported using the crude Lipson hematoporphyrin derivative to accomplish photoradiation therapy on human patients. The crude Lipson hematoporphyrin derivative has the ability to enter all kinds of cells and to be retained
15 in tumor cells after it has mostly cleared the serum. Subsequent irradiation with red light excites the crude Lipson derivative which in turn excites oxygen molecules. The excited oxygen molecules exist for a microsecond - long enough to attack tumor cell walls
20 and effect necrosis. In "Effects of Photo-Activated Porphyrins in Cell Surface Properties", Biochem Soc Trans 5:139-140, 1977, Kessel explained that cross-linking of proteins in tumor cell membranes causes leakage and eventual cell disruption.
25 The crude Lipson hematoporphyrin derivative enters normal tissue and causes unacceptable damage when therapeutic light sufficient to treat large



-1-

Description

PURIFIED HEMATOPORPHYRIN DERIVATIVE FOR
DIAGNOSIS AND TREATMENT OF TUMORS, AND METHOD

Specification

5 This application is a continuation-in-part of application Serial No. 424,647, filed September 27, 1982, entitled "Purified Hematoporphyrin Derivative for Diagnosis and Treatment of Tumors, and Method".

Background of the Invention

10 It is well known in the prior art to diagnose malignant tumors with photosensitive drugs. In "Etudes Sur Les Aspects Offerts Par Des Tumeur Experimentales Examinee A La Lumiere De Woods", CR Soc Biol. 91:1423-1424, 1924, Policard, the author, noted that some human and 15 animal tumors fluoresced when irradiated with a Wood's lamp. The red fluorescence was attributed to porphyrins produced in the tumor. In "Untersuchungen Uber Die Rolle Der Porphine Bei Geschwulstkranken Menschen Und Tieren", Z Krebsforsch 53:65-68, 1942, 20 Auler and Banzer showed that hematoporphyrin, a derivative of hemoglobin, would fluoresce in tumors but not in normal tissues following systemic injection into rats. In "Cancer Detection and Therapy. Affinity of Neoplastic Embryonic and Traumatized Regenerating 25 Tissue For Porphyrins and Metalloporphyrins", Proc Soc Exptl Biol Med. 68:640-641, 1948, Figge and co-workers demonstrated that injected hematoporphyrin would localize and fluoresce in several types of tumors induced in mice. In "The Use of a Derivative of Hematoporphyrin in Tumor Detection", J Natl Cancer Inst. 30



-4-

A still further object of the invention is to provide a novel method for the localization and/or treatment of tumors.

Other objects and advantages of the invention 5 will be apparent from the following description, taken in conjunction with the accompanying drawings:

Description of the Drawings

FIGURE 1 is a mass spectrometry print-out of the new drug;

10 FIGURE 2 is a visible light spectrum of the new drug in water solution;

FIGURE 3 and FIGURE 3A in combination illustrate an infra red spectrum of the new drug dispersed in potassium bromide;

15 FIGURE 4 is a carbon-13 nuclear magnetic resonance print-out of the new drug, referenced to dimethyl sulfoxide.

FIGURE 5 and FIGURE 5A in combination illustrate a print-out from a Waters Associates Variable 20 Wave Length Detector used in conjunction with its μ Bondpak C-18 column, showing various components including a peak formation representative of the new drug.

FIGURE 6 and FIGURE 6A in combination illustrate a print-out from a Waters Associates Variable 25 Wave Length Detector used in conjunction with its μ Bondpak C-18 column, showing the peak formation of the new drug, per se.

FIGURE 7 is a molecular formula depicting an ether, most likely the unit which associates to form 30 the high molecular weight aggregate of this invention.

FIGURE 8 and FIGURE 8A in combination illustrate a carbon-13 nuclear magnetic resonance print-out



-3-

tumors is applied. Severe edema and sloughing of healthy skin can occur when the crude Lipson derivative is used. Some patients are even harmed by exposure to ordinary sunlight thirty days following treatment with the drug. A better photosensitizer for treatment of tumors would have no systemic toxicity. The drug would have a better tissue distribution so it would clear the body faster. The prior art to applicants' knowledge does not disclose such a substance.

Summary of the Invention

The present invention relates to a new drug, obtained as a high molecular weight (i.e. greater than one porphyrin unit) aggregate derived from hematoporphyrin and purified by filtration through a micro-porous membrane, that selectively localizes in tumors, is fluorescent, and effects necrosis of said malignancies by light-induced reaction without material harm to normal tissue.

Accordingly, an object of the invention is to provide a photosensitive drug that can be used to effect photoradiation therapy of tumors.

A still further object of the invention is to provide a novel photosensitive drug that is highly tumor-selective, leaving normal tissue relatively unaffected after exposure to strong doses of therapeutic light.

A still further object of the invention is to provide a tumor-selective drug that fluoresces, delineating malignancy and aiding in diagnosis.

A further object of the invention is to provide a novel method of producing the above-identified drug.



-6-

is obtained. The powder may then be transferred to a 250 ML round bottom flask. The flask is then attached to a rotating evaporator and rotation under vacuum is maintained at room temperature for preferably 24 hours.

- 5 20.00 grams of the vacuum-dried powder is then preferably placed in a 4-liter aspirator bottle which may contain a magnetic stirring bar, and then 1000 ML of 0.1N sodium hydroxide is added thereto. This solution is preferably stirred for one hour and
- 10 1N hydrochloric acid is then added dropwise preferably using a buret. The 1N HCl is added until a pH of 7.0-7.4 is obtained and which is stable for 15 minutes. Using for instance the buret readings, the amount of sodium chloride produced from the neutralization of
- 15 sodium hydroxide by the hydrochloric acid may be calculated.

Isotonic solution is 0.9% NaCl or 9 grams NaCl per liter of solution. Therefore, the amount of NaCl produced during neutralization is subtracted from

- 20 the amount of NaCl required to make the solution isotonic. The calculated amount of NaCl is then added to the solution, and the solution is stirred for preferably 15 minutes. The quantity of solution should then be brought to a total volume of 4 liters by adding 0.9%
- 25 NaCl solution.

The aspirator bottle, containing the said solution, is then attached to transfer lines leading to a Milli-Pore Pellicon Cassette system fitted with a 10,000 molecular weight filter pack (Millipore Corporation, Bedford, Mass. 01730). It is preferable that the pH of the solution be 7.0-7.2 during this filtration process, and it is preferable that the temperature



-5-

of the new drug, referenced to tetramethylsilane in deuterated chloroform solvent. Magnification of the spectrum is shown in the ranges from 20-30 ppm and 55-75 ppm.

5 Description of Preferred Embodiment

(A) Preferred Preparation and Purification of the New Drug. (All equipment and reagents must be sterile.)

Add 285 ML of acetic acid to a 1000 ML Erlenmeyer flask containing a Teflon-coated magnetic stirring bar. Stir the acetic acid and slowly add 15 ML of concentrated sulfuric acid. Weigh out 15.0 grams of hematoporphyrin hydrochloride (preferably obtained from Roussel Corporation, Paris, France) and add said porphyrin to the acid solution. Stir for one hour.

Prepare a solution of 150 grams of sodium acetate in 3 liters of glass-distilled water using a 4-liter glass beaker. At the end of one hour, filter the porphyric-acid solution, preferably through Whatman No. 1 filter paper, allowing the filtrate to drip into the 4-liter beaker of 5% sodium acetate. The 5% sodium acetate solution now contains a dark red precipitate which is preferably allowed to stand for one hour with occasional stirring. The dark red precipitate is then again filtered, preferably using the above-identified filter mechanism. The filter cake from the filtering process is then washed with glass-distilled water until the filtrate is at pH 5.5-6.0 (1500-2500 ML of wash water may be required). The filter cake is then preferably allowed to dry in air at room temperature.

The air-dried precipitate is ground, using for instance, a mortar and pestle until a fine powder



-8-

in diameter, the mice were injected with a dose of 7.5 milligrams of the crude prior art Lipson derivative per kilogram of body weight for comparison purposes. Approximately 24 hours following the injection, the 5 tumor areas of the mice were shaved to remove the fur. The mice were exposed to red light (6000-7000A°) from an arc lamp at an intensity of 160 milliwatts per square centimeter for 30 minutes. Ten of twenty mice showed no apparent tumors seven days after treatment. The injected drug is retained in the tumor 10 cells longer as compared to normal tissue. This protocol was repeated using the new drug disclosed in this invention and equivalent results were obtained but using a drug dose of approximately only one-half (4 mg/kg 15 of body weight), as compared to the prior art Lipson drug. Red light is used in treating tumors by illuminating the same in order to take advantage of the ability of the longer visible wave lengths to penetrate tissue. Illumination may be accomplished by direct 20 illumination or by fiber optics, thus providing for illumination of any portion of the body generally without surgery.

In further tests ICR Swiss (Albino) mice were injected with a therapeutic dose of the crude 25 Lipson derivative (7.5 mg/kg of body weight). Approximately 24 hours following such injection, the hind feet of the mice were exposed to the same light conditions used in the aforescribed tumor response study. The damage to the hind feet was assessed as 2.0 on 30 an arbitrary scale where 0.0 is no damage and 5.0 is complete necrosis. Moist desquamation was evident and the foot area slowly returned to normal after about



-7-

of the solution be ambient. The Pellicon cassette system should preferably contain at least 25 liters of isotonic saline solution.

The peristaltic feed pump is turned on and the
5 solution is run through the Pellicon cassette system
at a pressure of preferably 10-20 p.s.i.g. Pressure
may be varied depending on the flow rate through the
system. Saline is added to the system to maintain a
volume of 4 liters in the associated aspirator bottle
10 containing the solution.

The filtration process is continued until
the solution contains substantially only the high
molecular weight, biologically active product. At
this time waste monomers are generally no longer pres-
15 ent. Exclusion of the waste through the microporous
membrane of the filter system is confirmed by analyz-
ing the high molecular weight, biologically active
product with a Bio-Gel P-10 column (obtainable for
instance from Bio-Rad, Richmond, Ca.) or by high per-
20 formance liquid chromatography using a μ Bondpak C-18
column with fixed variable wave length detector
(obtainable for instance from Waters Associates,
Milford, Ma.), as will be hereinafter described.

Concentrations of the product may be increased
25 by running the Pellicon cassette system without saline
feed. Concentrations of the product may be decreased
by adding saline solution. It is preferable that con-
centration of the new drug in solution is approximately
2.5 mg/cc.

30 (B) Animal Tests of the New Drug

DBA₂ Ha/D mice were transplanted with SMT-F
tumors. When the transplanted tumors reached 5-6mm



-10-

Elemental analysis of the disodium salt derivative of the new drug shows it to have an empirical formula of $C_{34} H_{35-36} N_4 O_{5-6} Na_2$, there being some uncertainty in hydrogen and oxygen due to traces of water which cannot be removed from the drug. A carbon-13 nuclear magnetic resonance study (FIGURE 4) of the drug in completely deuterated dimethylsulfoxide shows peaks at approximately 9.0 ppm for $-\text{CH}_3$, 18.9 ppm for $-\text{CH}_2-$, 24.7 ppm for CH_3CHOH , 34.5 ppm for $-\text{CH}_2-$, 62 ppm for CH_3CHOH , 94.5 ppm for =C (methine), 130-145 ppm for ring C, and 171.7 ppm for C ≡ O, all ppm being relative to dimethyl sulfoxide resonance at about 37.5 ppm. Additional vinyl peaks at approximately 118 and 127 ppm may be representative of the new drug or possibly a contaminant.

When the unfiltered reaction product described on page 7, lines 4-13 of this application, was eluted from a Waters Associates' Bondpak C-18 column using first, successively methanol, water and acetic acid (20:5:1) and then using tetrahydrofuran and water (4:1), four components were found. Three by-products were identified as hematoporphyrin, hydroxyethylvinyldeutero-porphyrin and protoporphyrin by comparison with standards on thin layer chromatography, with Rf values of approximately 0.19, 0.23, and 0.39 respectively (FIGURE 5) using Brinkman SIL silica plates and benzene-methanol-water (60:40:15) as elutent.

The fourth component shown in FIGURE 5A, was the biologically active drug of the invention. Chromatography (FIGURE 6 and FIGURE 6A) shows that exclusion of the above-identified impurities using the Milli-Pore Pellicon cassette system fitted with a 10,000 molecular



-9-

40 days. This protocol was repeated using the new drug disclosed in this application in doses of 4 mg/kg of body weight. Only slight erythema and/or edema was noticed following treatment. This condition dis-
5 appeared after 48-72 hours with no residual effects. This leads us to believe that skin photosensitivity may no longer be a significant problem when using this new drug.

(C) Analysis of the Drug

10 This new drug, as obtained from the Pellicon system, is a high molecular weight material derived by treating hematoporphyrin hydrochloride with acetic and sulfuric acids followed by appropriate hydrolysis. Its failure to pass through the Milli-Pore Pellicon

15 10,000 molecular weight filter pack indicates a molecular weight in excess of ten thousand. Mass spectrometry (FIGURE 1) of the new drug shows especially strong peaks at mass numbers of 149, 219, 591, 609 and characteristic but smaller peaks at 1200, 1218, 1290, 1809.

20 Spectrophotometry (FIGURE 2) of the new orange-red colored drug in aqueous solution reveals well-defined peaks at approximately 505, 537, 565 and 615 millimicrons.

Infrared spectrophotometry (FIGURE 3 and
25 FIGURE 3A) of the new drug disbursed in potassium bromide, reveals a broad peak associated with hydrogen stretching, said peak centered at approximately 3.0 microns, and a shoulder at approximately 3.4 microns. Finer peaks are observed at approximately 6.4, 7.1,
30 8.1, 9.4, 12 and 15 microns.



-12-

structure. In addition, many possible optical isomers of these structures exist.

A carbon-13 nuclear magnetic resonance study (FIGURES 8 and 8A) of the drug in deuterated chloroform

- 5 referenced to tetramethylsilane reveals two additional absorbances not previously apparent in FIGURE 4. Peaks at 24.7 ppm and 62 ppm in FIGURE 4 have shifted to 25.9 ppm and 65.3 ppm respectively in FIGURE 8A but newly-developed peaks at 27.9 ppm and 68.4 ppm in FIGURE 8A
10 represent resonances for CH_3 and H- $\text{C}-\text{OH}$ bonded from position 3- in FIGURE 8A, respectively. These newly-developed resonances substantiate the molecular formula depicted in FIGURE 7.

While tests using the new drug have been
15 performed to date on animals, it is believed that equivalent results would and will be obtained on humans, utilizing the same or less relative amount of drug to body weight. It is believed that the aforescribed treatment utilizing the drug of the invention, can be used
20 repeatedly without cumulative damage to normal tissues, providing that treatment is not overly aggressive.

While the aforementioned animal tests utilized a dosage of the new drug of approximately 4 mg/kg of body weight, in the treatment of the tumors in humans,
25 dosages as low as 1 mg/kg of body weight are believed effective utilizing the new drug. In any event dosages of the new drug of only approximately one-half of the necessary prior art dosages of the prior art related porphyrin drug, are equivalently effective in accomplishing necrosis of tumors.

Also, while the aforementioned animal tests utilized illumination one day following injection of



-11-

weight filter pack, has occurred, during processing of the drug of the invention.

The biologically active drug of this invention is probably an aggregate of ether molecules formed be-

- 5 tween two hematoporphyrin molecules by linkage of the hydroxyethylvinyl groups as shown in FIGURE 7. This linkage may occur through hydroxyethylvinyl groups in position 3- or 8- as numbered in FIGURE 7. Linkage may be achieved at position 3- in both halves of the ether,
10 at position 8- in both halves of the ether or through position 3- in one half of the ether and in position 8- in the other half of the ether.

These structures may be named as derivatives of ethyl ether, i.e.:

- 15 Bis -1- {3-(1-hydroxyethyl) deuteroporphyrin -8-yl} ethyl ether, as shown in FIGURE 7.
Other structured isomers may be named;
1- {3- (1-hydroxyethyl) deuteroporphyrin -8-yl} -1'
{8- (1-hydroxyethyl) deuteroporphyrin -3-yl} ethyl ether,
20 or
1- {8-(1-hydroxyethyl) deuteroporphyrin -3-yl} -1'
{3- hydroxyethyl) deuteroporphyrin -8-yl} ethyl ether,
and
Bis -1- {8- (1-hydroxyethyl) deuteroporphyrin -3-yl}
25 ethyl ether.

One or both hydroxyethyl groups at positions 3- or 8-, not used in ether formation, may dehydrate to form vinyl groups. Although experiments have not been conducted, experience indicates that ethers as shown in FIGURE 7

- 30 might be substituted with various combinations of hydrogen, alkyl groups, carboxylic acid groups and alcohol-containing groups at various locations of the



-14-

Claims

1. A substance effective for localizing and/or destroying tumors, said substance being fluorescent and photosensitive, and having the capability of localizing in and being retained in tumor cells as compared to normal tissues, said substance being a high molecular weight aggregate of a porphyrin derivative, said substance having adsorption peaks in the visible spectrum at approximately 505, 537, 565 and 615 millimicrons, said substance having adsorption peaks in the infrared spectrum at approximately 3.0, 3.4, 6.4, 7.1, 8.1, 9.4, 12 and 15 microns, said substance having adsorption peaks in carbon-13 nuclear magnetic resonance study at approximately 9.0, 18.9, 24.7, 34.5, 62, 94.5, 130-145, 171.7 ppm and possibly 118 and 127 relative to a 37.5 ppm resonance peak of dimethyl sulfoxide.
2. A substance in accordance with claim 1 wherein said substance shows mass numbers of 1899, 1866, 1809, 1290, 1200, 609, 591, 219 and 149 according to mass spectroscopy.
3. A substance in accordance with claim 1 wherein said substance is in liquid form having a concentration of approximately 2.5 mg/cc.
4. A substance in accordance with claim 3 wherein said liquid form includes isotonic saline solution at a pH of approximately 7.0 to 7.2.
5. A substance in accordance with claim 1 wherein the substance is orange-red in color.



-13-

the new drug, it is believed that a delay of up to seven days prior to illumination still will accomplish necrosis, and a time delay of two to four days between injection and illumination is generally preferable

5 in humans.

Furthermore, while an intensity of 160 mw/cm^2 for 30 minutes was utilized to activate the drug, it is believed that an intensity as high as 4000 mw/cm^2 for 20 minutes or as low as 5 mw/cm^2 for an extended period of 10 time may be utilized to accomplish necrosis. Less than 5 mw/cm^2 of illumination intensity will probably have no therapeutic effect, irrespective of time of application.

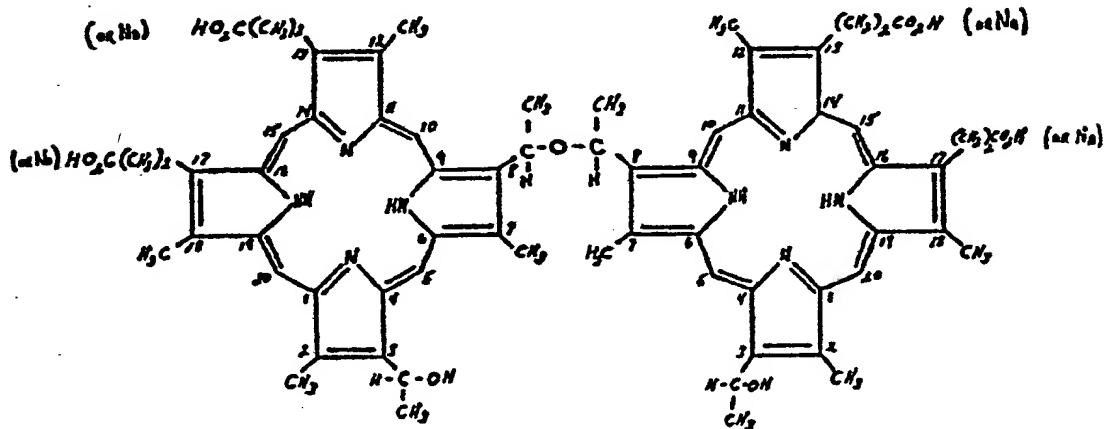
From the foregoing description, and accompanying drawings, it will be seen that the invention provides a new and novel drug, useful in the diagnosis and treatment of tumors, permitting utilization of reduced amounts of the drug as compared to related prior art drugs, and which results in less severe side 20 effects. The invention also provides a novel method of producing the new drug, together with a novel method of utilizing the drug in the treatment of tumors.

The terms and expressions which have been used are used as terms of description and not of limitation, and there is no intention in the use of such 25 terms and expressions of excluding any equivalents of any of the features shown or described, or portions thereof, and it is recognized that various modifications are possible within the scope of the invention 30 claimed.



-16-

12. A process in accordance with claim 11 wherein said substance is used in a dosage of from about 1 to 4 mg/kg of body weight of the host.
13. A process in accordance with claim 11 wherein the time delay between injection and illumination is within a range of about 1 to 7 days.
14. A process in accordance with claim 11 wherein said intensity of illumination is at least 5mw/cm² for an extended period of time, but no greater than 4000mw/cm² for twenty minutes.
15. A process in accordance with claim 11 wherein said substance is used in a dosage of about 4 mg/kg of body weight of the mouse, said intensity of illumination is about 160 mw/cm² for a period of about thirty minutes, the time delay between injection and illumination being approximately 24 hours.
16. A process in accordance with claim 11 wherein said illumination is red light having a wave length between 6000-7000A°.
17. A substance having the molecular formula



-15-

6. A substance in accordance with claim 1 wherein said substance has an empirical formula of approximately $C_{34} H_{35-36} N_4 O_{5-6} Na_2$.
7. A process for the production and purification
5 of the substance defined in claim 1, which comprises, reacting hematoporphyrin with acetic/sulfuric acids to form a solution, precipitating the crude product by neutralization in sodium acetate, dissolving the crude product with sodium hydroxide, adjusting the acidity of the solution to pH of 7.0-7.2 with hydrochloric acid, and passing the resultant impure solution through a porous membrane system to exclude low molecular weight by-products thereby effecting purification.
- 10 8. A process in accordance with claim 7 including adding sodium chloride to said impure solution to make the latter isotonic prior to passing it through said membrane system.
9. A process in accordance with claim 7 wherein said
20 substance in solution has a concentration of approximately 2.5 mg/cc and is adjusted to obtain said concentration by addition or removal of liquid.
10. A process in accordance with claim 7 wherein ambient temperatures are substantially maintained
25 throughout said process.
11. A process for the in vivo destruction of tumor cells using the substance of claim 1, which comprises injecting said substance into a host, waiting for a predetermined period of time, and
30 then illuminating the host with light at predetermined intensity.



-18-

21. A substance in accordance with claim 17 wherein the substance is orange-red in color.
22. A substance in accordance with claim 17 wherein said substance has an empirical formula of approximately $C_{68} H_{70} N_8 O_{11} (Na \text{ plus } H)_4$.
23. A process for the production and purification of the substance defined in claim 17, which comprises, reacting hematoporphyrin with acetic/sulfuric acids to form a solution, precipitating the crude product by neutralization in sodium acetate, dissolving the crude product with sodium hydroxide, adjusting the acidity of the solution to pH of 7.0-7.2 with hydrochloric acid, and passing the resultant impure solution through a porous membrane system to exclude low molecular weight by-products thereby effecting purification.
24. A process in accordance with claim 23 including adding sodium chloride to said impure solution to make the latter isotonic prior to passing it through said membrane system.
25. A process in accordance with claim 23 wherein said substance in solution has a concentration of approximately 2.5 mg/cc and is adjusted to obtain said concentration by addition or removal of liquid.
26. A process in accordance with claim 23 wherein ambient temperatures are substantially maintained throughout said process.



-17-

- effective for localizing and/or destroying tumors, said substance being fluorescent and photosensitive, and having the capability of localizing in and being retained in tumor cells as compared to normal tissues, said substance being a high molecular weight aggregate of a porphyrin derivative, said substance having adsorption peaks in the visible spectrum at approximately 505, 537, 656 and 615 millimicrons, said substance having adsorption peaks in the infrared spectrum at approximately 3.0, 3.4, 6.4, 7.1, 8.1, 9.4, 12 and 15 microns, said substance having adsorption peaks in carbon-13 nuclear magnetic resonance study at approximately 9.0, 18.9, 24.7, 34.5, 62, 94.5, 130-145, 171.7 ppm and possibly 118 and 127 relative to a 37.5 ppm resonance peak of dimethyl sulfoxide, said substance also having additional adsorption peaks in carbon-13 nuclear magnetic study at approximately 27.9 ppm and 68.4 ppm relative to the resonance peak of tetramethylsilane in deuterated chloroform solvent.
18. A substance in accordance with claim 17 wherein said substance shows mass numbers of 1899, 1866, 1809, 1290, 1200, 609, 591, 219 and 149 according to mass spectroscopy.
19. A substance in accordance with claim 17 wherein said substance is in liquid form having a concentration of approximately 2.5 mg/cc.
20. A substance in accordance with claim 19 wherein said liquid form includes isotonic saline solution at a pH of approximately 7.0 to 7.2.



-20-

34. An injectable drug dosage of approximately 1 to 4 mg/kg of body weight of the host and comprising a substance effective in said dosage amount for localizing and/or destroying tumors, said substance being fluorescent and photosensitive, and having the capability of localizing in and being retained in tumor cells as compared to normal tissues, said substance being a high molecular weight aggregate of a porphyrin derivative, said substance having adsorption peaks in the visible spectrum at approximately 505, 537, 565 and 615 millimicrons, said substance having adsorption peaks in the infrared spectrum at approximately 3.0, 3.4, 6.4, 7.1, 8.1, 9.4, 12 and 15 microns, said substance having adsorption peaks in carbon-13 nuclear magnetic resonance study at approximately 9.0, 18.9, 24.7, 34.5, 62, 94.5, 130-145, 171.7 ppm and possibly 118 and 127 relative to a 37.5 ppm resonance peak of dimethyl sulfoxide.
35. A drug dosage in accordance with claim 34 wherein activation of said drug after injection of said dosage into the host is accomplished by subjecting the host to illumination with red light having a wave length of between 6000-7000A° to activate said drug.



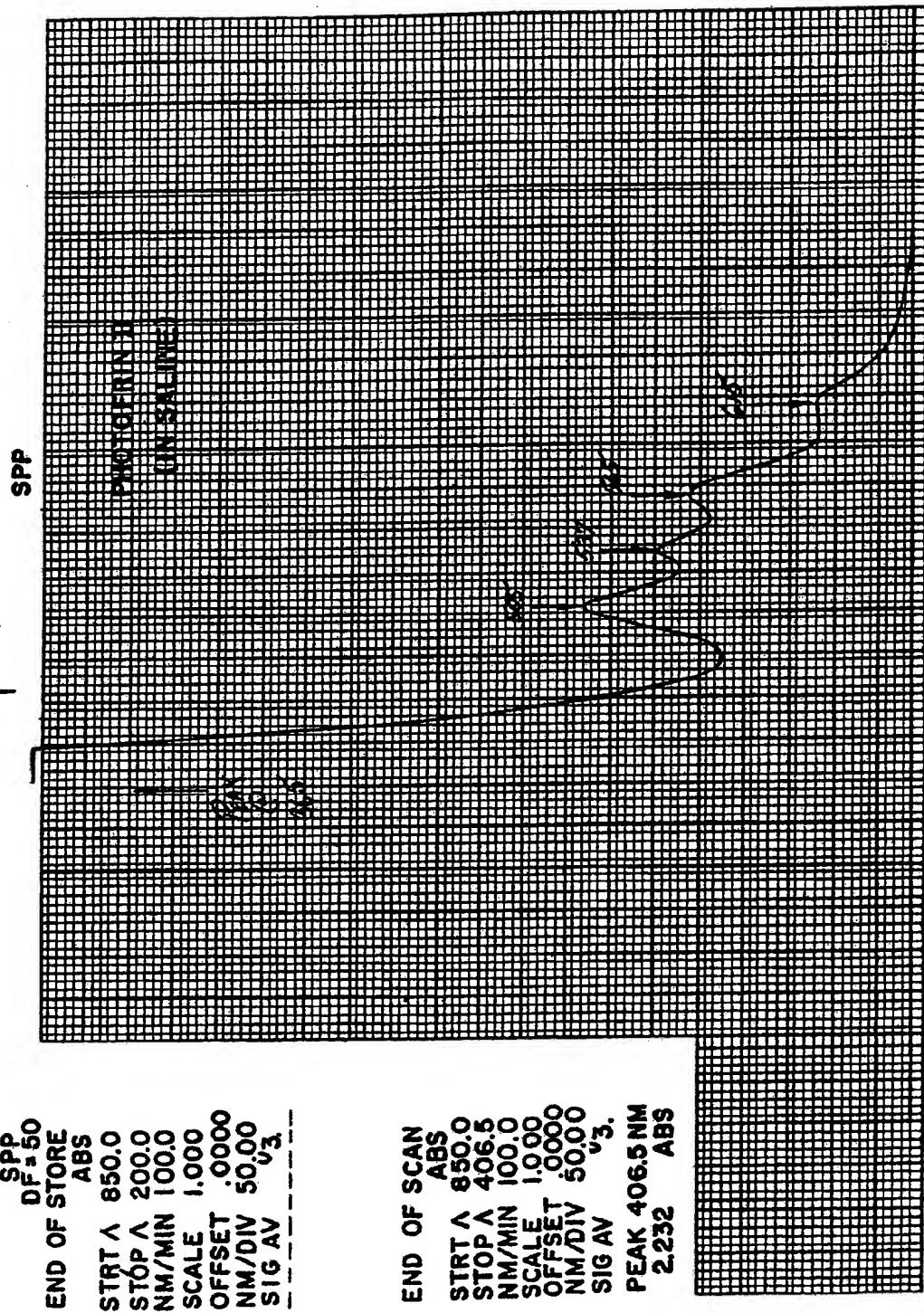
-19-

27. A process for the in vivo destruction of tumor cells using the substance of claim 17, which comprises injecting said substance into a host, waiting for a predetermined period of time, and then illuminating the host with light at predetermined intensity.
- 5
28. A process in accordance with claim 27 wherein said substance is used in a dosage of from about 1 to 4 mg/kg of body weight of the host.
- 10 29. A process in accordance with claim 27 wherein the time delay between injection and illumination is within a range of about 1 to 7 days.
- 15 30. A process in accordance with claim 27 wherein said intensity of illumination is at least 5mw/cm^2 for an extended period of time, but no greater than 4000mw/cm^2 for twenty minutes.
- 20 31. A process in accordance with claim 27 wherein said substance is used in a dosage of about 4 mg/kg of body weight of the mouse, said intensity of illumination is about 160mw/cm^2 for a period of about thirty minutes, the time delay between injection and illumination being approximately 24 hours.
- 25 32. A process in accordance with claim 27 wherein said illumination is red light having a wave length between $6000-7000\text{\AA}$.
- 30 33. A substance in accordance with claim 17 which is substituted with one or more hydrogen, alkyl groups, carboxylic acid groups and alcohol-containing groups at various locations of the structure.

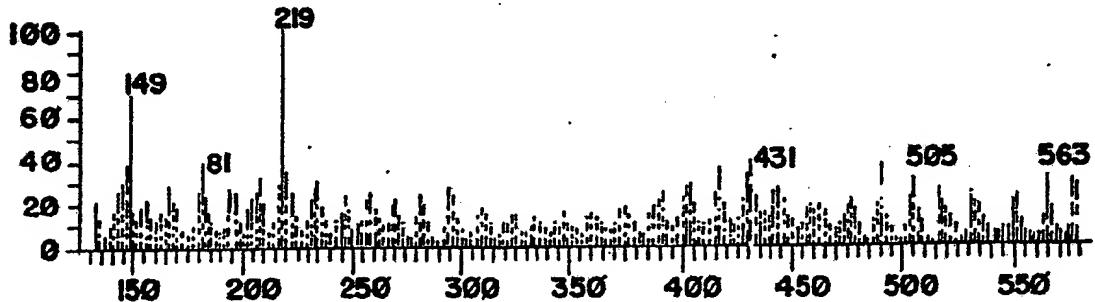
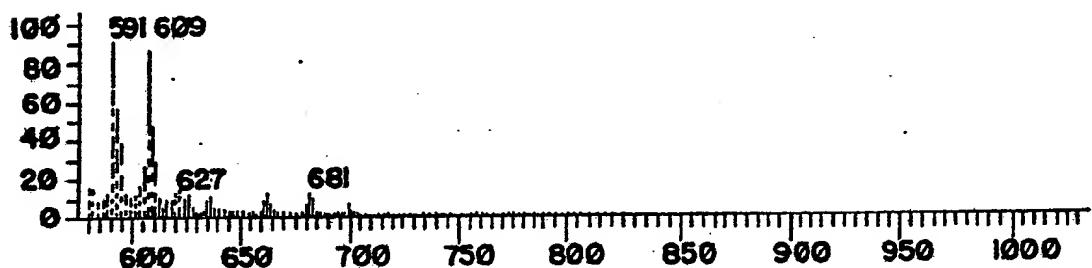
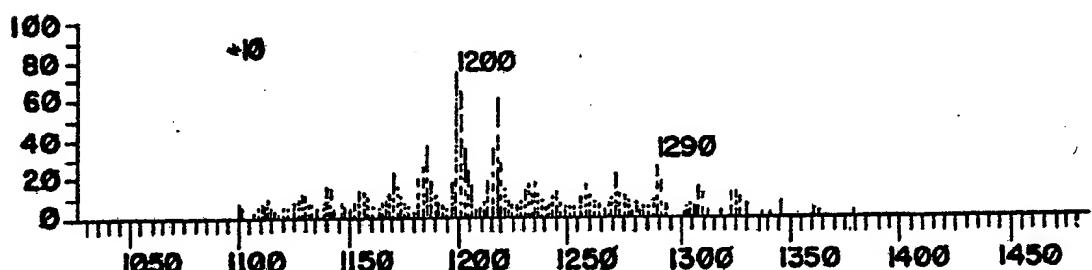
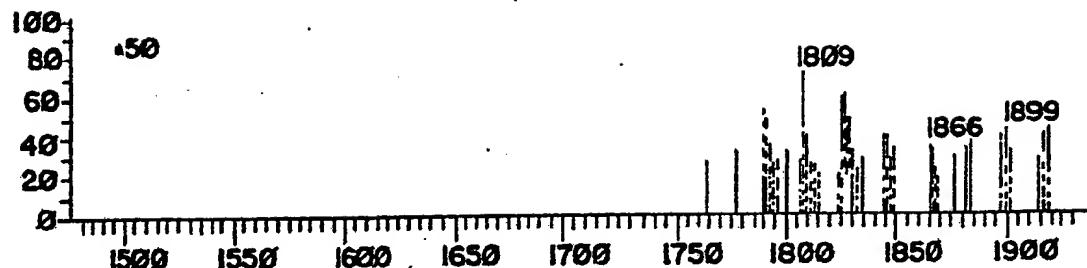


2 / 12

FIG 2



1 / 12



F 1 1



4 / 12

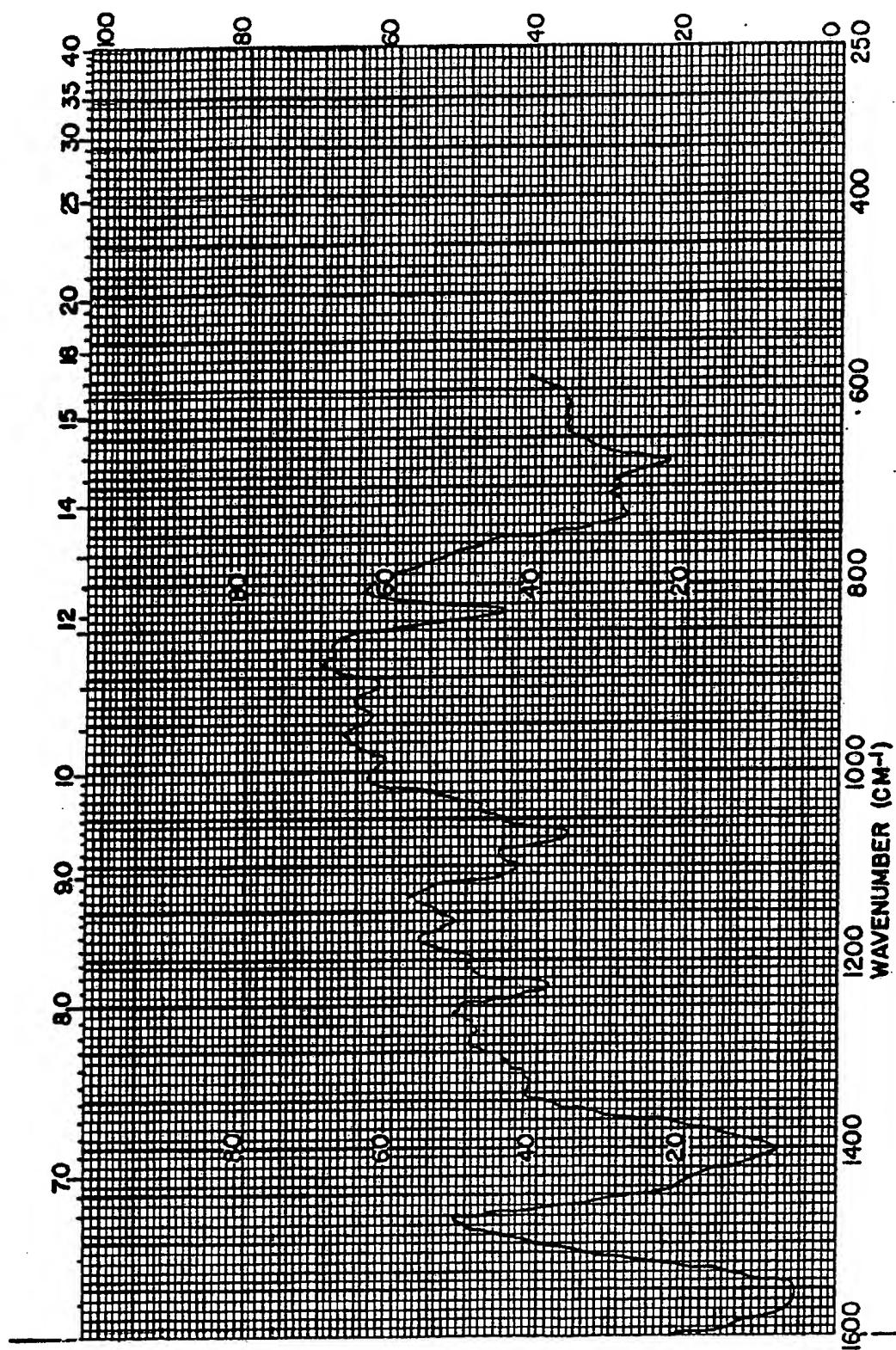


FIG 2 A

S / 12

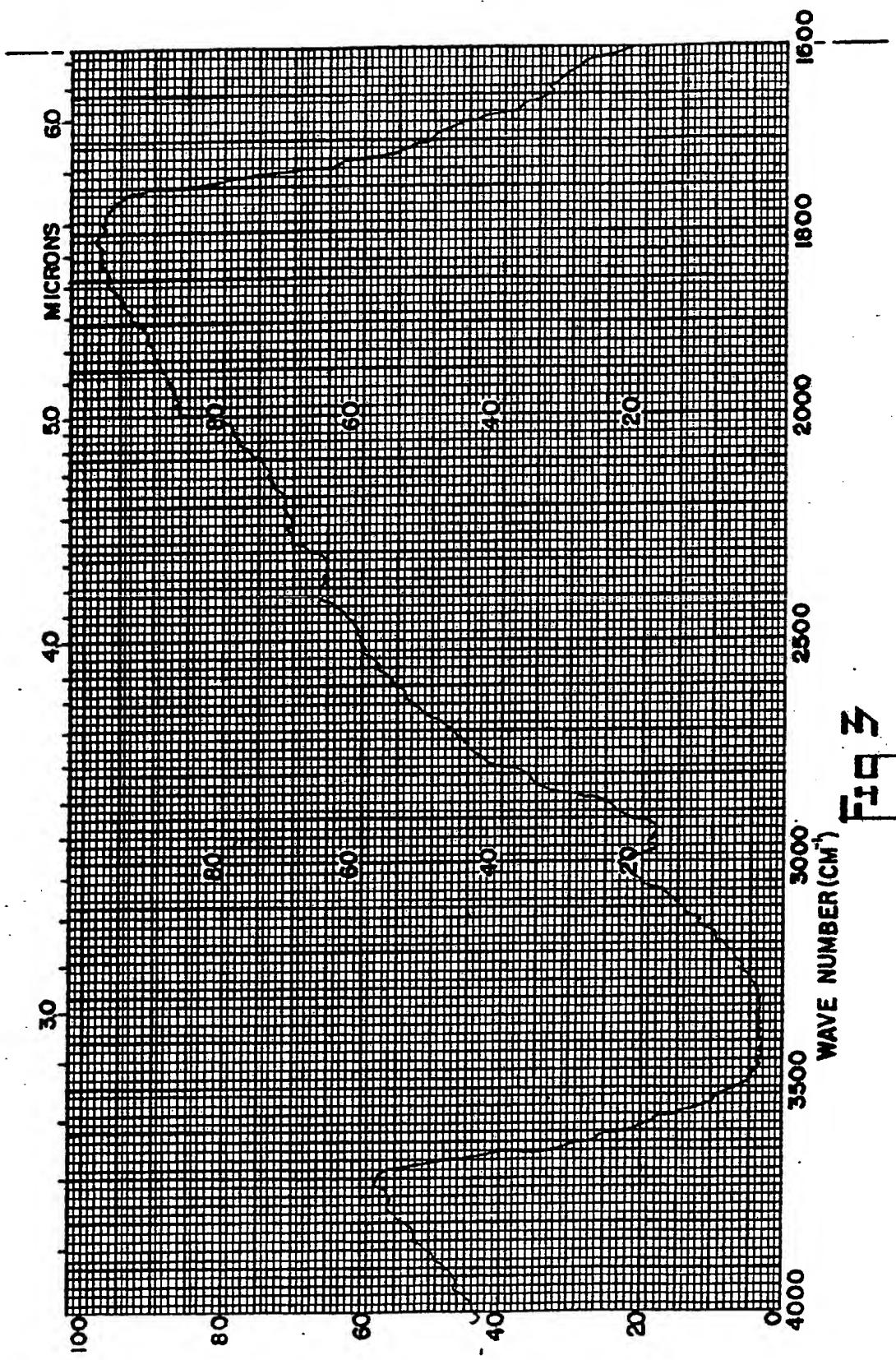
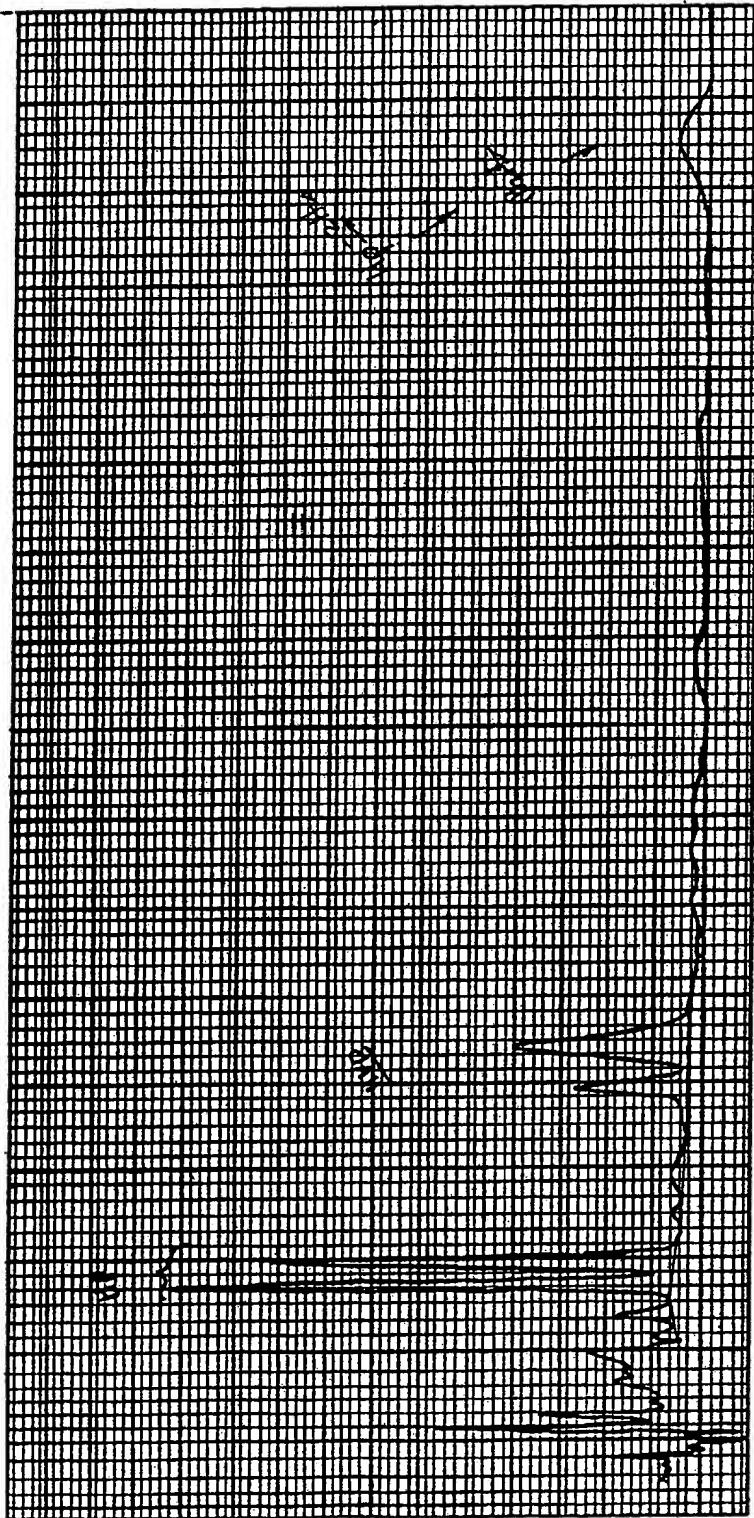


Fig 7

6 / 12

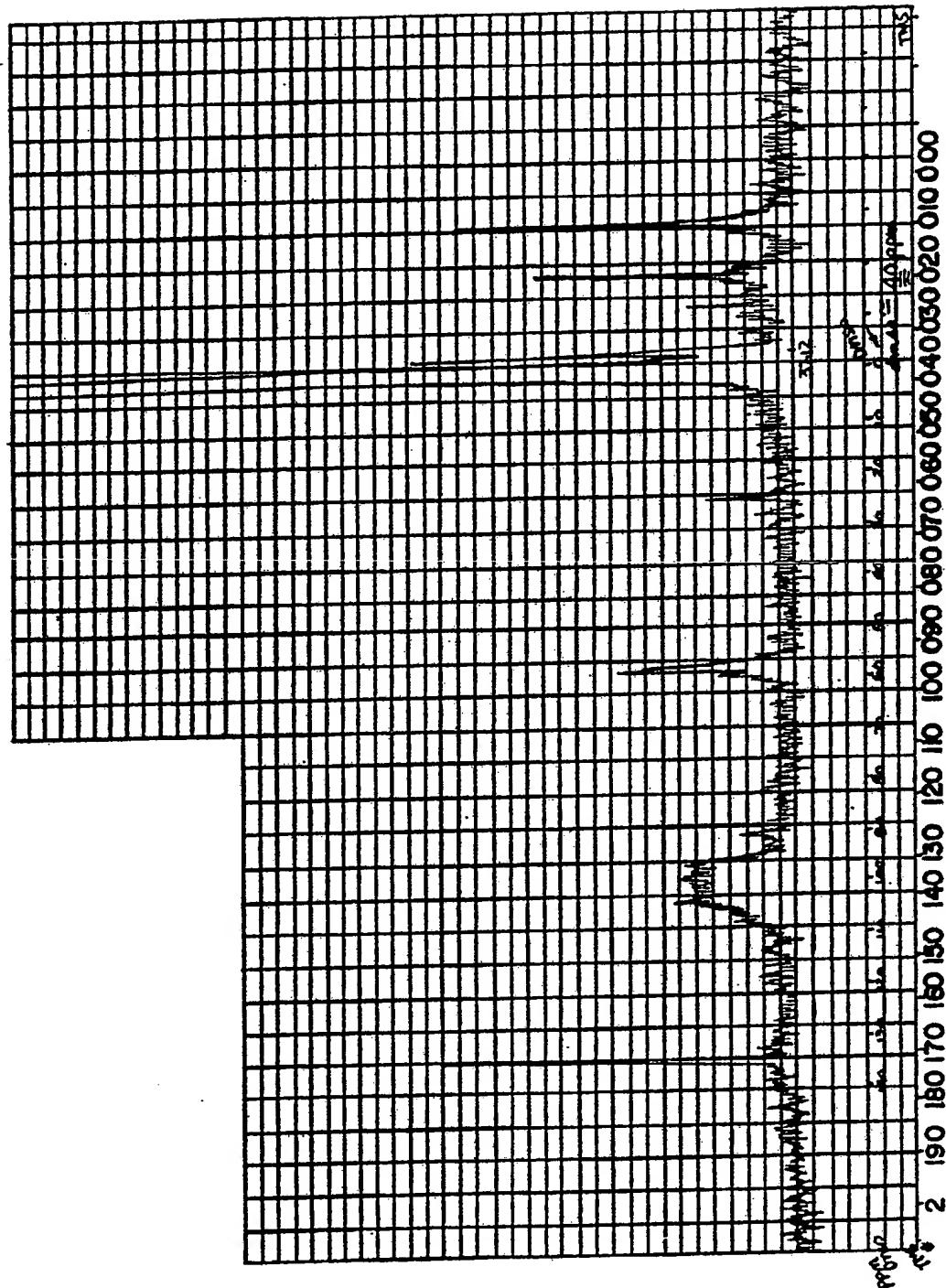


695

69



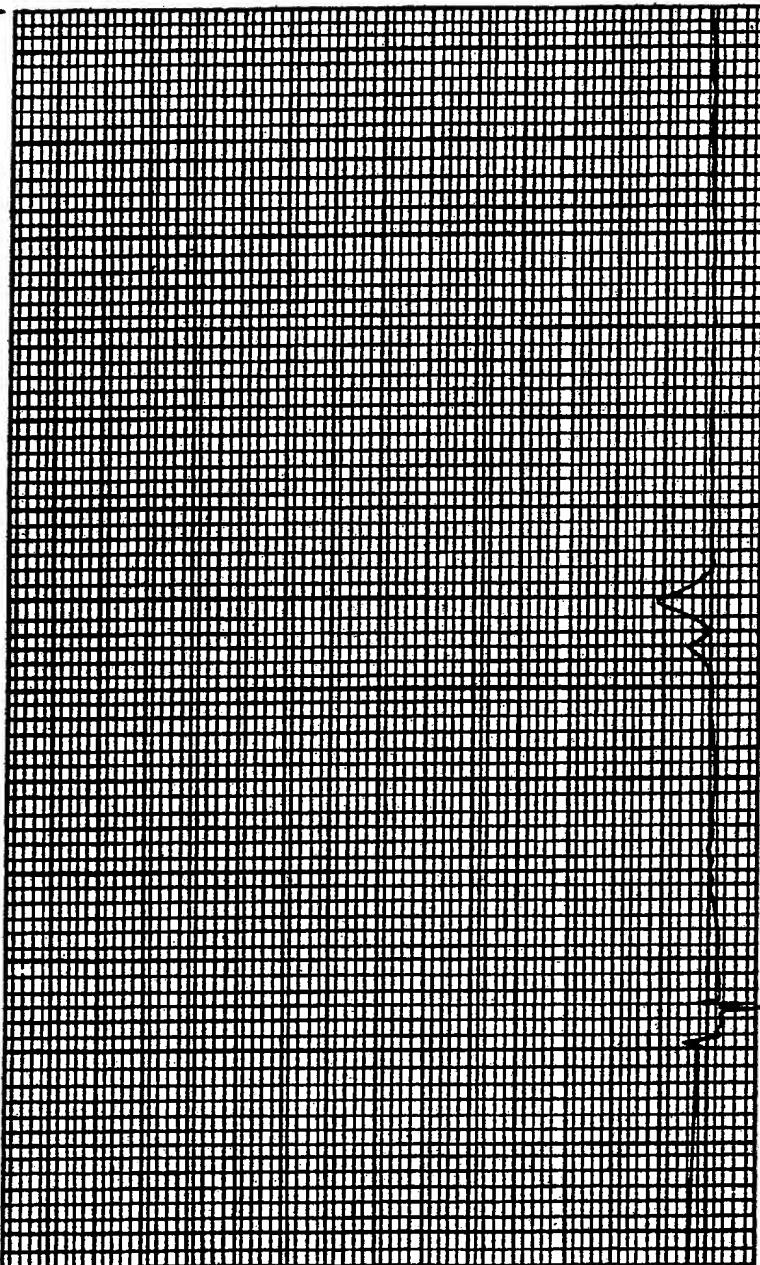
5 / 12



四
五



8 / 12



E95
E96



7 / 12

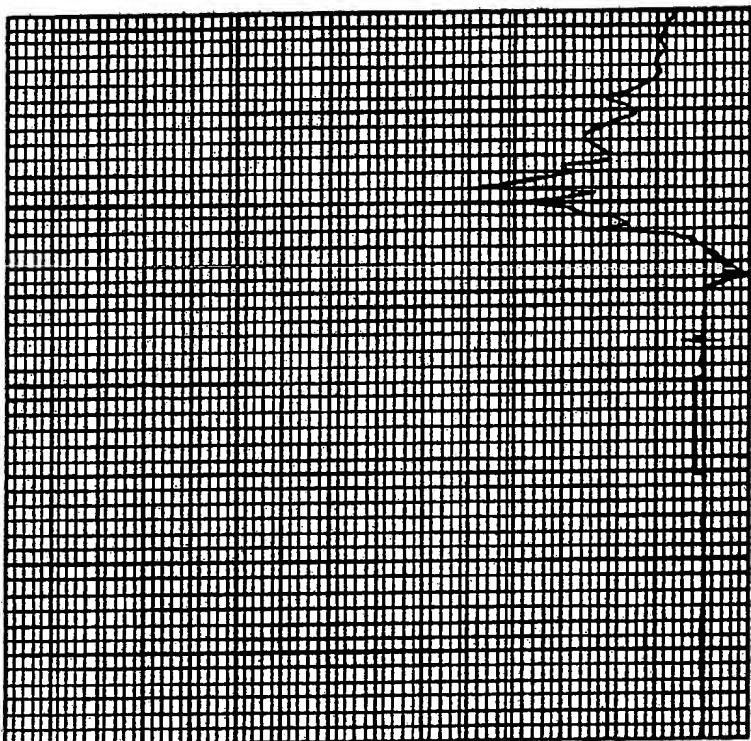
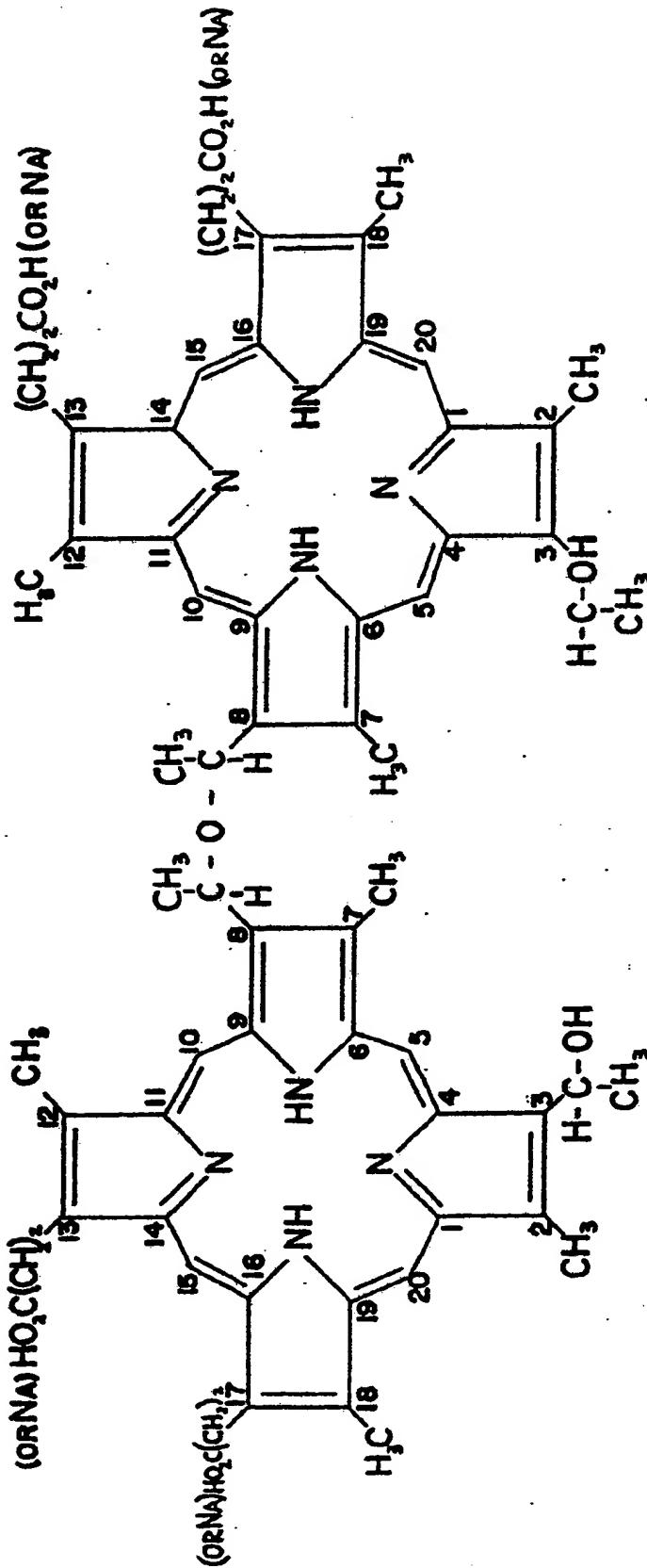
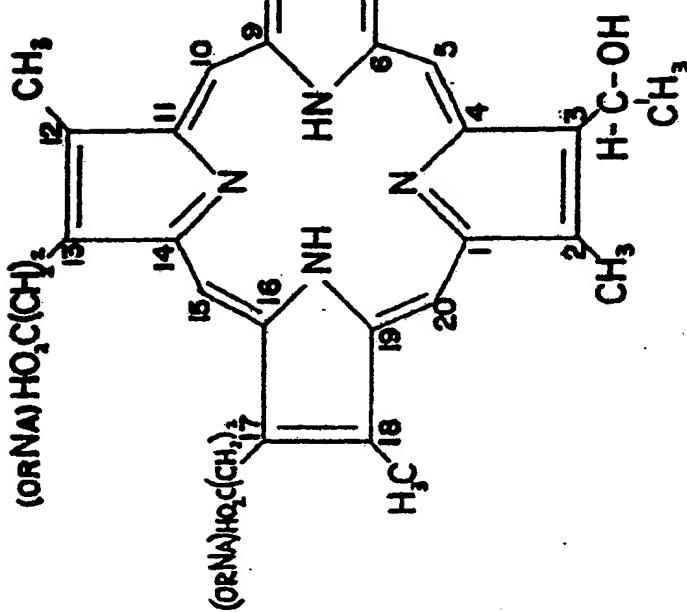


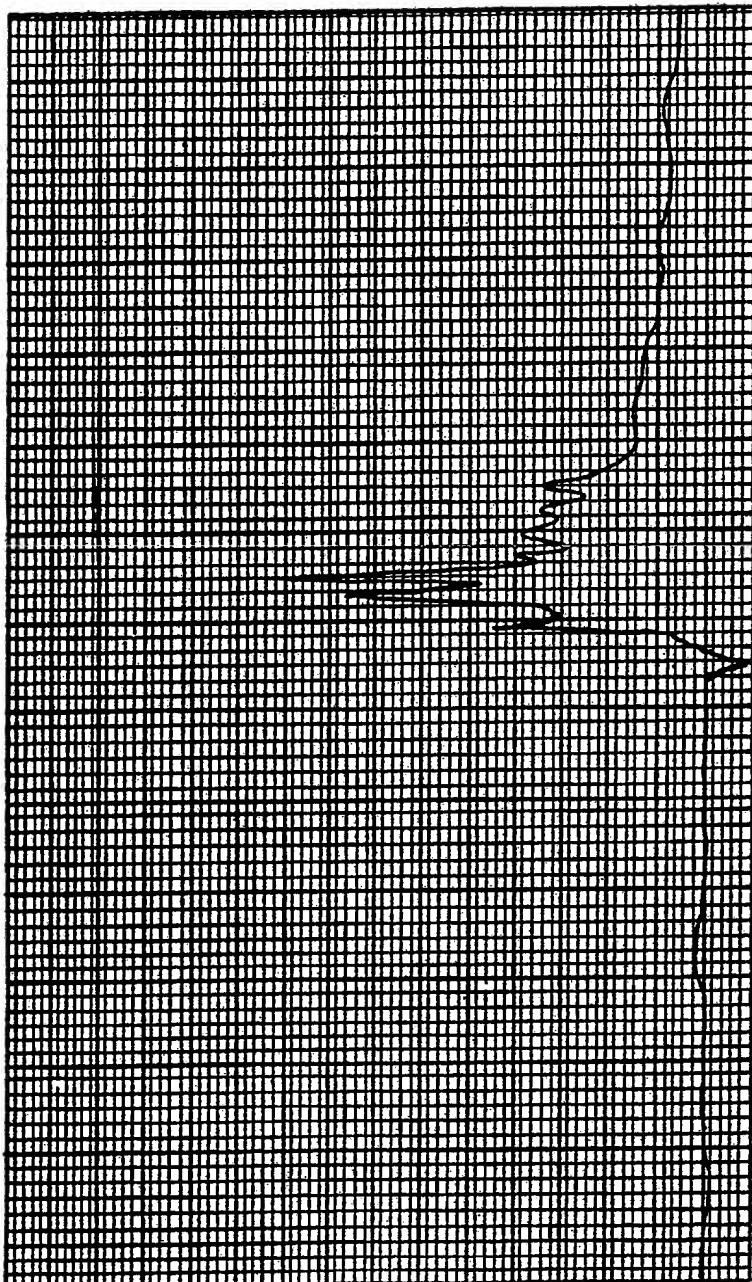
FIG 5A
[Small square box]



10712

7
49

9 / 12

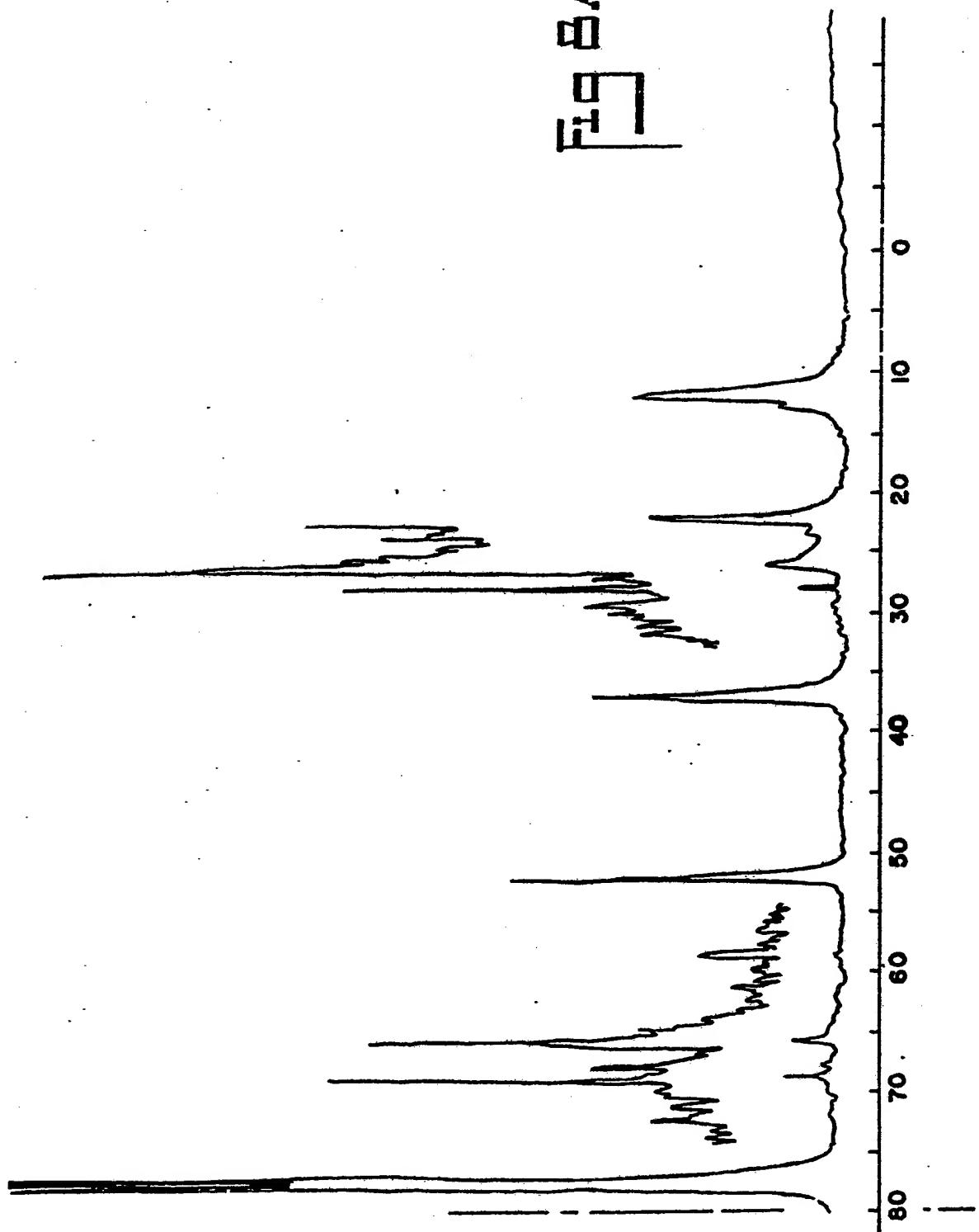


EQ 6A
[]

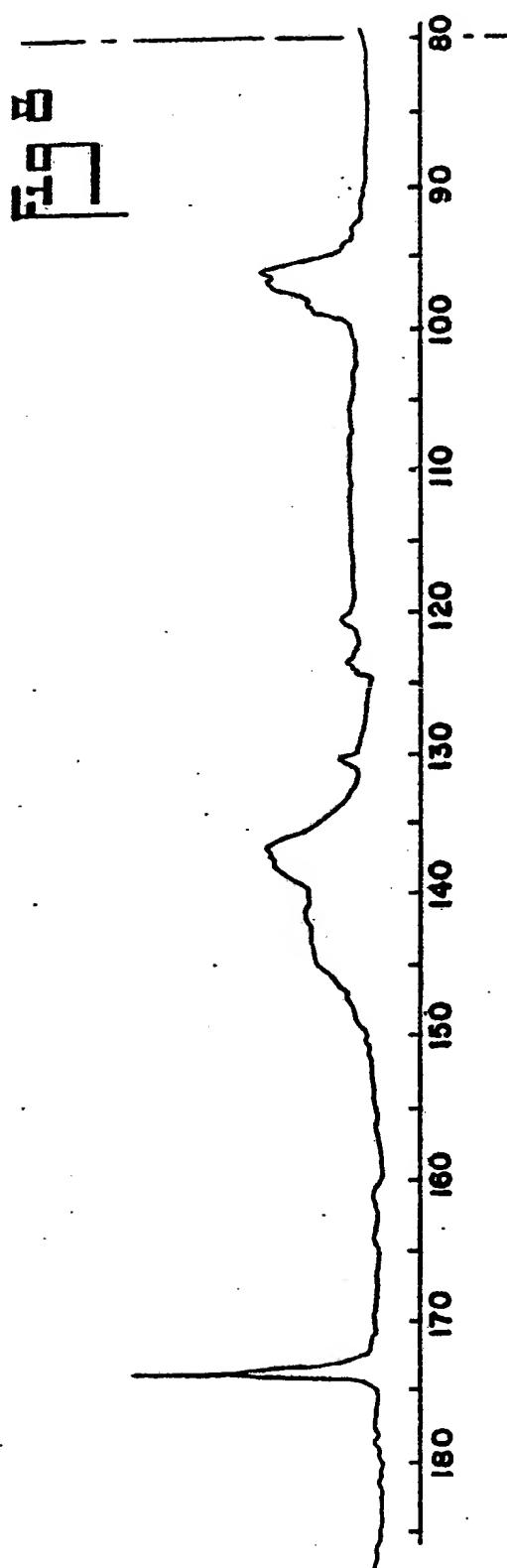


12 / 12

Fig 8A



11 / 12



INTERNATIONAL SEARCH REPORT

International Application No PCT/US83/01379

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ¹⁸

According to International Patent Classification (IPC) or to both National Classification and IPC
 Int. Cl. C07D 209/58; A61K 31/40
 U.S. Cl. 260/326.33; 424/274

II. FIELDS SEARCHED

Minimum Documentation Searched ¹⁹

Classification System	Classification Symbols
U.S.	260/326.33; 424/274

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ²⁰

Chemical Abstracts 1907 - 1982 "Hematoporphyrin"

III. DOCUMENTS CONSIDERED TO BE RELEVANT ²¹

Category ²²	Citation of Document, ²³ with indication, where appropriate, of the relevant passages ²⁴	Relevant to Claim No. ²⁵
X	DOUGHERTY ET AL., Journal National Cancer Institute, Vol. 62, pp. 231-37 (1979)	1-35
X	DOUGHERTY ET AL., Cancer Research, Vol. 38 pp. 2628-2635, (1978)	1-35
X	LIPSON ET AL., Journal National Cancer Institute, Vol. 26, pp 1-8 (1961)	1-35

* Special categories of cited documents: ¹⁸

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"Z" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search ²⁶

5 December 1983

Date of Mailing of this International Search Report ²⁷

08 DEC 1983

International Searching Authority ²⁸

ISA/US

Signature of Authorized Officer ²⁹

THIS PAGE BLANK (USPTO)

SUPPLEMENTARY
EUROPEAN SEARCH REPORT

Application number

EP 83 90 3067

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
			TECHNICAL FIELDS SEARCHED (Int. Cl. 4)
X	CHEMICAL ABSTRACTS, vol. 97, no. 1, July 5, 1982, page 394, abstract 3930s, COLUMBUS, OHIO (US), SHILIN XU et al.: "Hematoporphyrin sodium, as a chemotherapeutic agent", & Zhongcaoyao 1981, 12(8), 343-4. * the whole abstract *	1-35	C 07 D 209/58 A 61 K 31/40
X	--- CHEMICAL ABSTRACTS, vol. 78, no. 11, March 19, 1973, page 212, abstract 68960d, COLUMBUS, OHIO (US), D.R. SANDERSON et al.: "Hematoporphyrin as a diagnostic tool. Preliminary report of new techniques". & Cancer (Philadelphia) 1972, 30(5), 1368-72. * the whole abstract *	1-35	
P,X	--- CHEMICAL ABSTRACTS, vol. 98, no. 9, February 28, 1983, page 293, abstract 68090h, COLUMBUS, OHIO (US), C.J. GOMER et al.: "Hematoporphyrin derivative photoradiation therapy for the treatment of intraocular tumors; examination of acute normal ocular tissue toxicity". & Cancer Res. 1983, 43(2), 721-7. * the whole abstract *	1-35	C 07 D 487/00 C 07 D 519/00
The supplementary search report has been drawn up for the claims attached hereto.			
Place of search THE HAGUE		Date of completion of the search 19-12-1984	Examiner MAISONNEUVE J.A.
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			
T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

THIS PAGE BLANK (USPTO)

-14-

Claims

1. A substance effective for localizing and/or destroying tumors, said substance being fluorescent and photosensitive, and having the capability of localizing in and being retained in tumor cells as compared to normal tissues, said substance being a high molecular weight aggregate of a porphyrin derivative, said substance having adsorption peaks in the visible spectrum at approximately 505, 537, 565 and 615 millimicrons, said substance having adsorption peaks in the infrared spectrum at approximately 3.0, 3.4, 6.4, 7.1, 8.1, 9.4, 12 and 15 microns, said substance having adsorption peaks in carbon-13 nuclear magnetic resonance study at approximately 9.0, 18.9, 24.7, 34.5, 62, 94.5, 130-145, 171.7 ppm and possibly 118 and 127 relative to a 37.5 ppm resonance peak of dimethyl sulfoxide.
2. A substance in accordance with claim 1 wherein said substance shows mass numbers of 1899, 1866, 1809, 1290, 1200, 609, 591, 219 and 149 according to mass spectroscopy.
3. A substance in accordance with claim 1 wherein said substance is in liquid form having a concentration of approximately 2.5 mg/cc.
4. A substance in accordance with claim 3 wherein said liquid form includes isotonic saline solution at a pH of approximately 7.0 to 7.2.
5. A substance in accordance with claim 1 wherein the substance is orange-red in color.



THIS PAGE BLANK (USPTO)

-15-

6. A substance in accordance with claim 1 wherein said substance has an empirical formula of approximately C₃₄ H₃₅₋₃₆ N₄ O₅₋₆ Na₂.
7. A process for the production and purification
5 of the substance defined in claim 1, which comprises, reacting hematoporphyrin with acetic/sulfuric acids to form a solution, precipitating the crude product by neutralization in sodium acetate, dissolving the crude product with sodium
10 hydroxide, adjusting the acidity of the solution to pH of 7.0-7.2 with hydrochloric acid, and passing the resultant impure solution through a porous membrane system to exclude low molecular weight by-products thereby effecting purification.
- 15 8. A process in accordance with claim 7 including adding sodium chloride to said impure solution to make the latter isotonic prior to passing it through said membrane system.
9. A process in accordance with claim 7 wherein said
20 substance in solution has a concentration of approximately 2.5 mg/cc and is adjusted to obtain said concentration by addition or removal of liquid.
10. A process in accordance with claim 7 wherein ambient temperatures are substantially maintained
25 throughout said process.
11. A process for the in vivo destruction of tumor cells using the substance of claim 1, which comprises injecting said substance into a host, waiting for a predetermined period of time, and
30 then illuminating the host with light at predetermined intensity.



THIS PAGE BLANK (USPTO)

-16-

12. A process in accordance with claim 11 wherein said substance is used in a dosage of from about 1 to 4 mg/kg of body weight of the host.

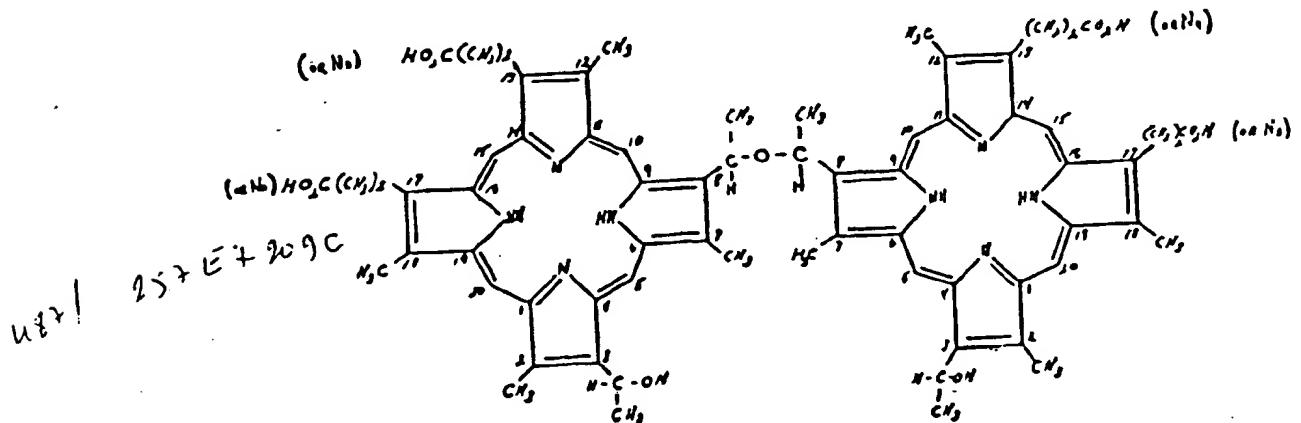
13. A process in accordance with claim 11 wherein the time delay between injection and illumination is within a range of about 1 to 7 days.

14. A process in accordance with claim 11 wherein said intensity of illumination is at least 5mw/cm² for an extended period of time, but no greater than 4000mw/cm² for twenty minutes.

15. A process in accordance with claim 11 wherein said substance is used in a dosage of about 4 mg/kg of body weight of the mouse, said intensity of illumination is about 160 mw/cm² for a period of about thirty minutes, the time delay between injection and illumination being approximately 24 hours.

16. A process in accordance with claim 11 wherein said illumination is red light having a wavelength between 6000-7000A°.

17. A substance having the molecular formula



THIS PAGE BLANK (USPTO)

-17-

- effective for localizing and/or destroying tumors, said substance being fluorescent and photosensitive, and having the capability of localizing in and being retained in tumor cells as compared to normal tissues, said substance being a high molecular weight aggregate of a porphyrin derivative, said substance having adsorption peaks in the visible spectrum at approximately 505, 537, 656 and 615 millimicrons, said substance having adsorption peaks in the infrared spectrum at approximately 3.0, 3.4, 6.4, 7.1, 8.1, 9.4, 12 and 15 microns, said substance having adsorption peaks in carbon-13 nuclear magnetic resonance study at approximately 9.0, 18.9, 24.7, 34.5, 62, 94.5, 130-145, 171.7 ppm and possibly 118 and 127 relative to a 37.5 ppm resonance peak of dimethyl sulfoxide, said substance also having additional adsorption peaks in carbon-13 nuclear magnetic study at approximately 27.9 ppm and 68.4 ppm relative to the resonance peak of tetramethylsilane in deuterated chloroform solvent.
18. A substance in accordance with claim 17 wherein said substance shows mass numbers of 1899, 1866, 1809, 1290, 1200, 609, 591, 219 and 149 according to mass spectroscopy.
19. A substance in accordance with claim 17 wherein said substance is in liquid form having a concentration of approximately 2.5 mg/cc.
20. A substance in accordance with claim 19 wherein said liquid form includes isotonic saline solution at a pH of approximately 7.0 to 7.2.



THIS PAGE BLANK (USP)

-18-

21. A substance in accordance with claim 17 wherein the substance is orange-red in color.
22. A substance in accordance with claim 17 wherein said substance has an empirical formula of approximately $C_{68} H_{70} N_8 O_{11} (Na \text{ plus } H)_4$.
23. A process for the production and purification of the substance defined in claim 17, which comprises, reacting hematoporphyrin with acetic/sulfuric acids to form a solution, precipitating the crude product by neutralization in sodium acetate, dissolving the crude product with sodium hydroxide, adjusting the acidity of the solution to pH of 7.0-7.2 with hydrochloric acid, and passing the resultant impure solution through a porous membrane system to exclude low molecular weight by-products thereby effecting purification.
24. A process in accordance with claim 23 including adding sodium chloride to said impure solution to make the latter isotonic prior to passing it through said membrane system.
25. A process in accordance with claim 23 wherein said substance in solution has a concentration of approximately 2.5 mg/cc and is adjusted to obtain said concentration by addition or removal of liquid.
26. A process in accordance with claim 23 wherein ambient temperatures are substantially maintained throughout said process.



THIS PAGE BLANK

-19-

27. A process for the in vivo destruction of tumor cells using the substance of claim 17, which comprises injecting said substance into a host, waiting for a predetermined period of time, and
5 then illuminating the host with light at predetermined intensity.
28. A process in accordance with claim 27 wherein said substance is used in a dosage of from about 1 to 4 mg/kg of body weight of the host.
- 10 29. A process in accordance with claim 27 wherein the time delay between injection and illumination is within a range of about 1 to 7 days.
30. A process in accordance with claim 27 wherein
15 said intensity of illumination is at least 5mw/cm² for an extended period of time, but no greater than 4000mw/cm² for twenty minutes.
31. A process in accordance with claim 27 wherein
20 said substance is used in a dosage of about 4 mg/kg of body weight of the mouse, said intensity of illumination is about 160mw/cm² for a period of about thirty minutes, the time delay between injection and illumination being approximately 24 hours.
32. A process in accordance with claim 27 wherein
25 said illumination is red light having a wave length between 6000-7000A°.
33. A substance in accordance with claim 17 which is substituted with one or more hydrogen, alkyl groups, carboxylic acid groups and alcohol-containing groups at various locations of the
30 structure.



THIS PAGE BLANK (USPTO)

-20-

34. An injectable drug dosage of approximately 1 to 4 mg/kg of body weight of the host and comprising a substance effective in said dosage amount for localizing and/or destroying tumors, said substance being fluorescent and photosensitive, and having the capability of localizing in and being retained in tumor cells as compared to normal tissues, said substance being a high molecular weight aggregate of a porphyrin derivative, said substance having adsorption peaks in the visible spectrum at approximately 505, 537, 565 and 615 millimicrons, said substance having adsorption peaks in the infrared spectrum at approximately 3.0, 3.4, 6.4, 7.1, 8.1, 9.4, 12 and 15 microns, said substance having adsorption peaks in carbon-13 nuclear magnetic resonance study at approximately 9.0, 18.9, 24.7, 34.5, 62, 94.5, 130-145, 171.7 ppm and possibly 118 and 127 relative to a 37.5 ppm resonance peak of dimethyl sulfoxide.
35. A drug dosage in accordance with claim 34 wherein activation of said drug after injection of said dosage into the host is accomplished by subjecting the host to illumination with red light having a wave length of between 6000-7000A° to activate said drug.



THIS PAGE BLANK (USPTO)